

Lipid Metabolism on the Perfused Dog Lung

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The lipolytic activity of the isolated lung perfusion fluid and lung tissue was demonstrated and also the activity of whole animal plasma was measured. After perfusion, the lipid composition of both lung tissue and fatty acid composition of phospholipid subfraction were investigated. The activity of lung tissue was reduced after perfusion. From force fed dogs, the lipolytic activity of perfusion fluid and lung tissue was higher than that from fasted dogs. The dog lung injected with fibrin clots showed a reduction of the lipolytic activity and the simultaneously collected plasma lipolytic activity was decreased in comparison with that of normal dogs. It was proved that the dextran infused dog lung had lipolytic activity and these substances had a heparinlike action. Furthermore, free fatty acid was produced into the perfusion fluid. On the otherhand, the analysis of lung lipid contents, phospholipid fraction was reduced following the reduction of the rate of choline in the dextran infused lungs.

In the fatty acid composition of choline in the postperfusion lung tissue from dextran infused dogs, the amount of palmitic acid was decreased. These findings suggest that the lung perform an active lipid metabolism and the lipoprotein lipase may be situated in the capillary bed surface. The results from the analysis of lipid composition of lung tissue in this study may be related to the surface active agents.

INTRODUCTION

Since HAHN¹⁾ had discovered in 1943 that the alimentary lipemia was cleared rapidly in dogs by the intravenous injection of heparin, many studies^{2), 3), 4), 5)} on the clearing factor were performed. ANDERSON and FAWCETT⁶⁾ and BROWN, BOYLE and ANFINSEN⁷⁾ demonstrated the appearance of a lipolytic enzyme in the blood after the injection of heparin in vivo and in vitro, which hydrolysed artificial triglyceride emulsion. This enzyme differed from pancreatic lipase from the studies on various activators and inhibitors and was named clearing factor lipase. On the otherhand, KORN^{8), 9)} had demonstrated the same lipolytic enzyme in the tissue and named it lipoprotein lipase, because the enzyme hydrolysed only the protein bounded triglyceride or chylomicron. This enzyme is not present in normal blood but appears in the

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absorption of lipids.

In spite of many efforts, this enzyme has not been obtained in pure form. On the perfusion studies of hearts ¹⁰⁾ or limbs ¹¹⁾ using the fluid containing heparin and plasma albumin, the clearing factor was released rapidly in the perfusion fluid and these results have supported that the enzyme localized on the vascular wall might be easily liberated into the blood stream by combining with heparin. This enzyme catalyses the hydrolysis of the triglyceride moiety of chylomicrons and low density β -lipoproteins. After the tissue lipoprotein lipase was first determined in the rat hearts by KORN^{8),9)} in the extracts of adipose tissue ^{12),13),14),15)}, mammary gland ¹⁶⁾, lung ^{17),18)} and arterial blood vessel wall⁸⁾, the same enzyme was demonstrated. In vivo, orally administered exogenous fats are absorbed by the intestine and carried to the lung through the thoracic duct. The lung is the first important organ to be reached by the fats and this anatomical characteristic suggests that the lung may play an important role in lipid metabolism. There is a report that the lung has the function of taking up the circulating triglycerides and hydrolyses. LOCHNER¹⁹⁾ reported that the lung released free fatty acid, because the free fatty acid level was higher in the arterial blood than simultaneously drawn mixed venous blood in man and dog.

SCHRADE²⁰⁾ reported that the lung had the ability of uptake of lipid in rats after oral administration of olive oil. Also it is clinically recognized that pulmonary tuberculosis is often accompanied by a fatty liver and pneumonia ^{21),22)}. Lung cancer and tuberculosis cause lipemia ^{23),24),25),26)}. In the present study, for the exclusion of the influence of other organs, especially of the liver, the isolated dog lung was perfused and the lipolytic activity of perfusion fluid, lung tissue and also plasma of whole animal was measured. The lipid composition of postperfused lung tissue was compared with unperfused lung tissue. Furthermore, the phospholipid subfraction and its fatty acid composition were analysed.

EXPERIMENTAL MATERIALS AND METHODS

(A) *Treatment of Animals (Table 1)*

Healthy mongrel dogs of both sexes maintained on a normal kennel diet and weighing between 7 to 12 Kg were used. The animals were divided into three groups.

Group 1. Five animals were only fasted overnight before the experiment and three other animals were fed forcibly 50 ml of ethyl-linoleate through a stomach tube under intravenous sodium pentobarbital anesthesia (30–60 mg when necessary) after fasting overnight. The latter animals were used six hours after ethyl-linoleate administration. This

Table 1
Distribution of Animals

Group of Animals		Number of animals		
		Perfusion fluid	Plasma	Lung Tissue
1. Intact	Fasted	5	3	3
	Force Fed	3	3	3
2. Fibrin Clots Injection	Fasted	3	3	3
	Force Fed	5	3	3
3. Dextran Infusion	Fasted	3	3	3
	Force Fed	4	3	3

group was used for the control.

Group 2. In eight animals, pulmonary vascular lesions were produced by fibrin clots injection following the procedure of BARNARD²⁷⁾. Namely 20 ml of dog blood was collected aseptically and allowed to clot. The serum was discarded and the clots were homogenized and washed in sterile saline until they were buff to pink in color and suspended in 20 ml of sterile saline. This clot suspension was injected intravenously four times at a week interval. In these animals, three were fasted and the other five were fed forcibly ethyl-linoleate six hours before the experiment.

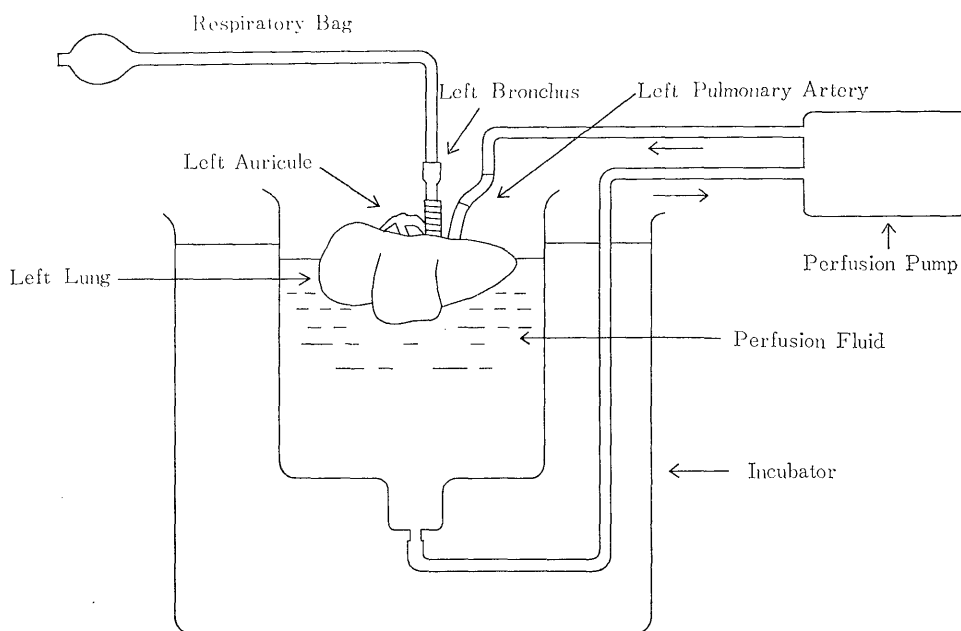
Group 3. In seven animals, rapid intravenous infusion of dextran (6% dextran (high molecular weight) in 5% glucose solution) was performed by the procedure of Said²⁸⁾. Infusion rate was 30–40 ml per minute. In each animal, 0.5 and 30 minutes after the beginning of infusion, 5 ml of blood was collected and used for determination of lipoprotein lipase. In these animals, three were fasted, and the other four were fed forcibly ethyl-linoleate six hours before experiment. In group 2 pulmonary vascular lesions and in group 3 lung edema were demonstrated histologically.

(B) *Perfusion Technique*

The left lung of the experimental animals was isolated following the technique of WEST²⁹⁾. The animal was anesthetized with sodium pentobarbital and fixed on the operating table on the right lateral position. The thoracotomy was done via a left fourth or fifth intercostal incision. After the pulmonary ligament was incised, the left pulmonary artery and the left bronchus were isolated and incised. Finally the left auricle was incised. The left lung was then removed and the pulmonary artery and left auricle were washed with 50 ml of 1/10M oxalated acid. Immediately

after washing, the glass cannula filled with perfusion fluids was tied into the left pulmonary artery and then the left bronchus was tied into the another glass cannula for respiration. Diagram of perfusion was shown in Fig. 1. This procedure was finished within five minutes after

Fig. 1 Diagram of Perfusion



the removal of the lung. Perfusion was carried out by T-N type micro-perfusion pump made by NATUME Industry. Perfusion rate was 40 ml per minute. Respiration was established manually and the rate of respiration was 16–20 per minute. The perfusion fluid consisted of 0.1 vol. of 20% Fatgen, one vol. of dog oxalated plasma, one vol. of phosphate buffer (pH 8.0) and one vol. of 0.9% saline.

(C) Enzyme Sample

(1) Plasma. After drawing five ml of blood, sodium heparin (10u per Kg body weight) was injected into foreleg vein and 3,5,10,30 and 60 minutes after the injection, each five ml of blood was drawn with 1/10 vol. of 1/10 M oxalated acid and immediately chilled at 4°C in the ice box. Each sample was centrifuged for 10 minutes at 4,000 rpm in 0°C and the supernatant layer was used as a specimen.

(2) Lung Tissue. Lung tissues from right and left lungs (in which the left lung was perfused) weighing exactly 500 mg were placed into the glass homogenizer added two ml of physiological saline solution. After homogenizing them in the ice bath, the homogenate was centrifuged for 10 minutes at 4,000 rpm in 0°C. One ml of each supernatant layer was obtained for the measurement of lipolytic activity.

(3) Perfusion Fluid. The fluid was used as an enzyme sample,

(D) *Assay of Lipolytic Activity*³⁰⁾

The substrate contained 0.1 vol. of 20% Fatgen (sesame oil emulsion), one vol. of dog oxalated plasma (heated at 56°C for five minutes and filtrated), one vol. of phosphate buffer (pH 8.0), and one vol. of 0.9% saline. The substrate was preincubated at 37°C for 30 minutes before use. Immediately after mixing one ml of the sample of lung tissue or plasma was placed into 1.5 ml of the substrate, one ml of the mixture was placed into DOLE's³¹⁾ extraction mixture and the remaining mixture was incubated at 38°C for 30 minutes. One ml of the perfusion fluid was immediately added into DOLE's³¹⁾ extraction mixture and the remaining fluid was incubated at 38°C for 30 minutes. One ml of each incubated mixture was also placed into DOLE's³¹⁾ mixture. Measurement of free fatty acid was followed by DOLE's method.³¹⁾ One ml of the sample was added into the glass stoppered tube with five ml of DOLE's³¹⁾ extraction mixture (40 parts by volume of isopropyl alcohol, 10 parts heptane, and one part 1 N H₂SO₄) and shaken vigorously for 10 minutes. After standing 10 minutes, 3 ml of heptane and two ml of water (CO₂ free) were introduced into the mixture, and then shaken vigorously for five minutes. Three ml of the upper heptane layer was transferred into a conical centrifugal tube containing one ml of DOLE's³¹⁾ titration mixture (0.01% thymol blue and 90% ethanol in water) and then titrated with 0.018 N sodium hydroxide using a microburette. A stream of nitrogen was delivered to the bottom of the test tube and forced carbon dioxide from the sample. In all the experiments, palmitic acid standard and the heptane layer adding no sample as a blank were similarly titrated. A lipolytic activity was represented as the differences of free fatty acid concentration in the mixture before and after incubation. The nitrogen content of lung tissue homogenates were also measured by Micro-Kjeldahl method.

(E) *Extraction of Lipid*

Lipids from lung tissue were extracted by the method of Folch and LEES³²⁾. Total lipid was measured by the gravimetry. Triglyceride was measured by the method of VAN-HANDEL and ZILVERSMIT³³⁾, phospholipid by the method of ALLEN³⁴⁾, and total cholesterol by the method of ZAK³⁵⁾ and HENLY³⁶⁾. Analysis of phospholipid subfractions were accomplished by the modified microquantitative method of KATES³⁷⁾.

(F) *Gas Liquid Chromatography*

Separation of the extracted lipids into simple lipid and phospholipid was performed by thin layer chromatography. Methylesterification of fatty acid was done by STOFFEL's³⁸⁾ method. Analytical condition of gas liquid chromatography is shown in Table 2. The identification of each fatty acid was performed by the retention time³⁹⁾. Quantity of each fatty acid was calculated by the weight of curves, and expressed as percentage of the total weight.

Table 2
Analytical Condition of Gas Liquid Chromatography

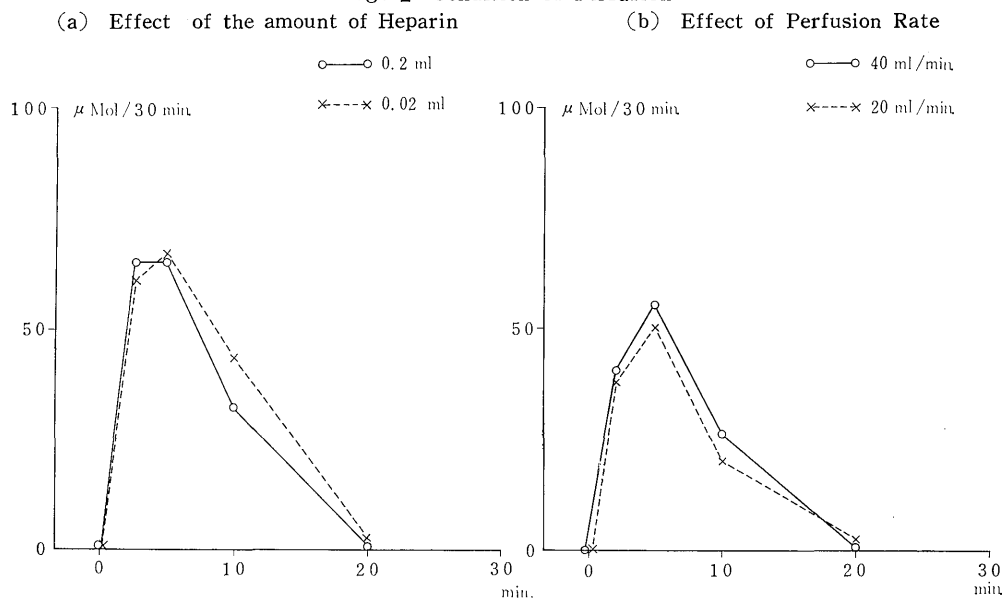
Apparatus SHIMADZU GC-IB
 Colum Diethyleneglycol Succinate 15% on Shimadzeite
 Support mesh 60-80
 I.D. 4 mm Length 3 m
 Column Temperature 200°C
 Sample Temperature 280°C
 Detector Temperature 240°C
 Carrier Gas He Flow Rate 30 ml/min. Pressure 3.0kg/cm²
 Detector Hydrogen Flame Ionization Detector (Type HFD-1)
 H₂ Flow 70ml/min. Air Flow 700ml/min.

RESULTS

(A) Condition of the Perfusion

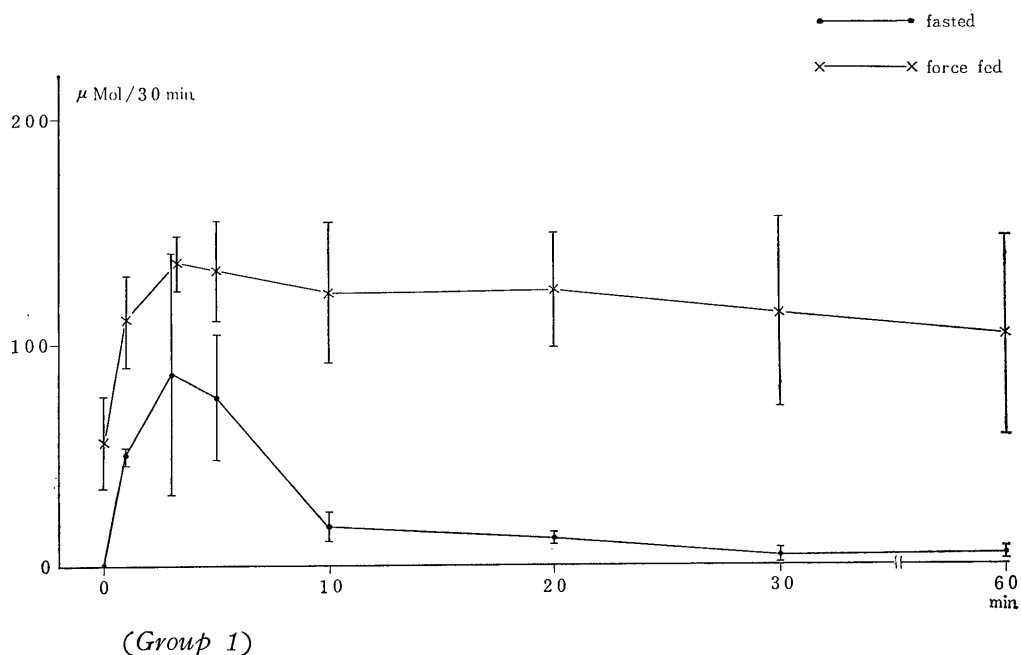
(a) Heparin Concentration (Fig. 2-a). In the amounts of heparin added through the perfusion system, (varying 0.02-0.2ml) no change was seen on the activity of lipoprotein lipase released into the perfusion fluid and so in this study 0.2 ml of heparin was added to every experiment.

Fig. 2 Condition of Perfusion



(b) Perfusion Rate (Fig. 2-b). In comparison with the perfusion rate between 20 and 40 ml per minute, no marked difference was seen in the activity of lipoprotein lipase. In this experiment, the perfusion rate was performed in 40 ml per minute.

(B) Lipolytic Activity of Perfusion Fluid from the Normal Dogs

Fig. 3 Lipolytic Activity of the Perfusion Fluid (Group 1)

(a) Dogs Fasted for 24 Hours (Fig. 3). Five minutes after starting the perfusion, heparin was introduced through the perfusion system. The lipolytic activity increased rapidly, and was maximum in three minutes after the introduction of heparin, and fell off rapidly. 30 minutes after adding heparin, the activity was similar to the level before the heparin was introduced.

(b) Force Fed Dogs (Fig. 3). The same experiment was carried out six hours after the administration of ethyl-linoleate. The lipolytic activity was present before adding heparin. After adding heparin, the activity markedly increased and reached a maximum in three minutes after the starting of perfusion. Its maximum rate was 1.6 times higher than in fasted animals and then stayed at a plateau level. This figure differed significantly from that of the fasting dogs.

(C) *Lipolytic Activity of Lung Tissue in Intact Dogs (Fig. 4)*

(Group 1)

(a) Dogs Fasted for 24 Hours. Lipolytic activity in the left lung tissue (used in the perfusion study) was lower than that in the unperfused right lung tissue.

(b) Force Fed Dogs. Lipolytic activity in the right lung tissue was significantly higher than that in the fasted animals, but in the left tissue lipolytic activity was markedly decreased. This decrease of lipolytic activity suggested that lipoprotein lipase was released into the perfusion fluids.

(D) *Plasma Lipolytic Activity in Vivo of the Control Dogs (Fig. 5)*

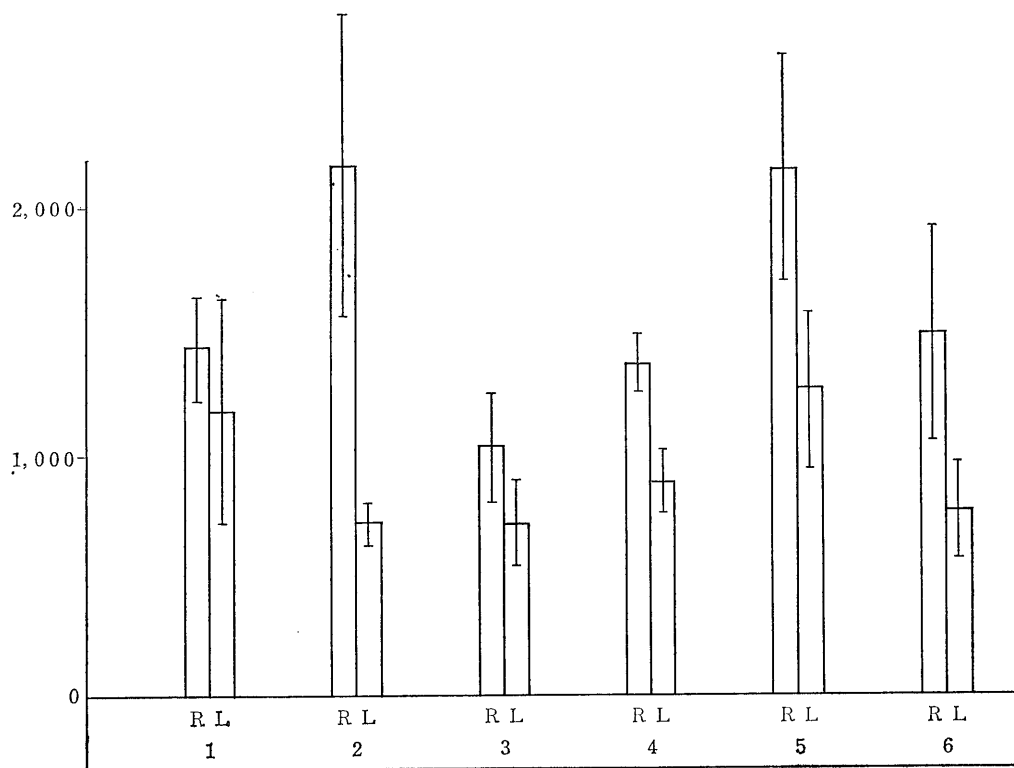


Fig. 4 Lipolytic Activity of the Lung Tissue

R: Right Lung L: Left Lung (1) fasted (2) force fed (3) clots-fasted
(4) clots-force fed (5) dextran-fasted
(6) dextran-force fed

Values expressed as Mol/100 mg Nitrogen/30 min.

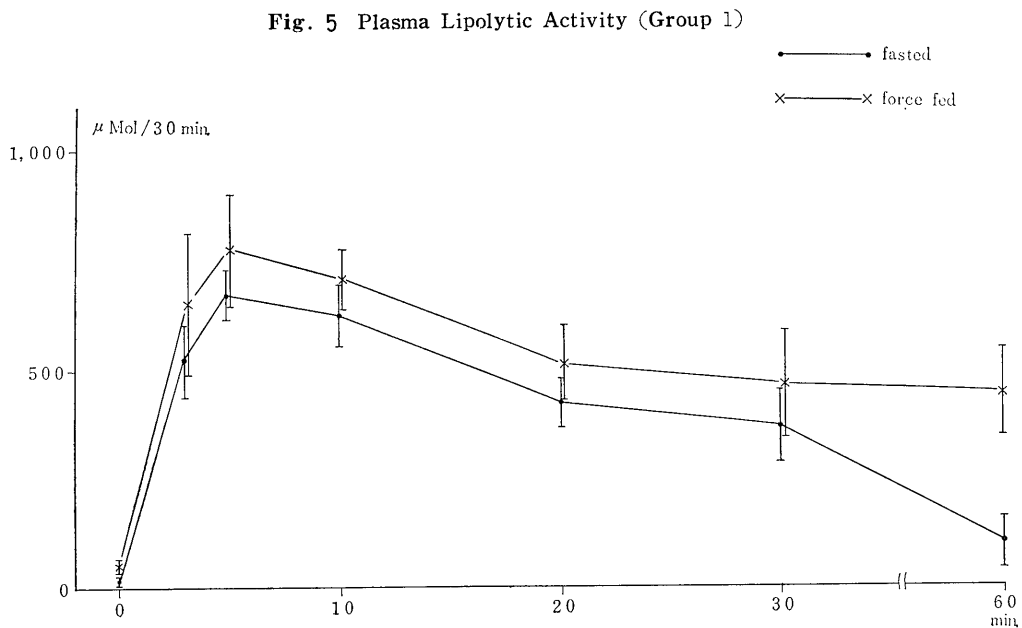


Fig. 5 Plasma Lipolytic Activity (Group 1)

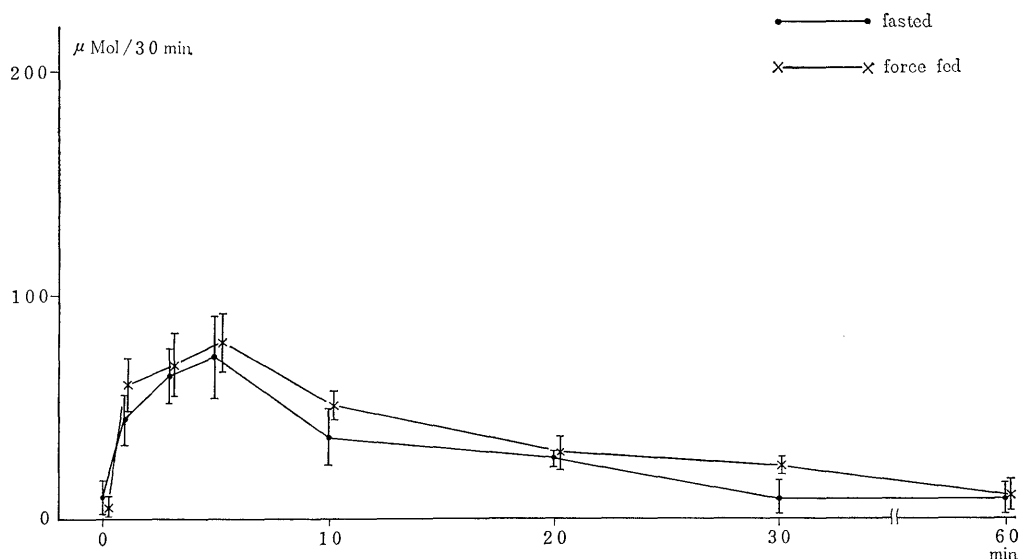
Intravenous injection of heparin caused the increase of lipolytic activity in plasma. In the fasted state, maximum rate was present five minutes after the injection of heparin, and the activity was significantly decreased 60 minutes after. On the contrary, in force fed animals the maximum value was higher than that in fasted animals and was present 60 minutes after the injection. The increased lipolytic activity might be due to the administration of ethyl-linoleate.

(E) *Alteration of Lipolytic Activity in Lung of Fibrin Clots Injected Dogs*

(Group 2)

(a) Lipolytic Activity of Perfusion Fluids (Fig. 6). In the fasted animals, the lipolytic activity was elevated but the maximum value was present in five minutes after adding heparin and this elevation was differed from that of intact animals. In force fed animals, the

Fig. 6 Lipolytic Activity of the Perfusion Fluid (Group 2)

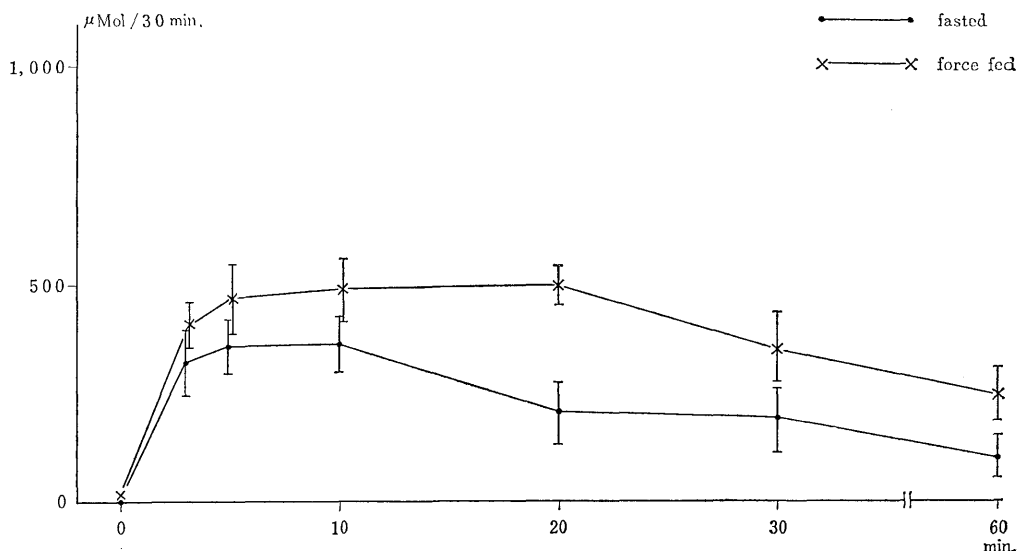


increasing rate of the activity was not so high as that in intact force fed animals. This fact may be due to the effects of the alteration of the lung capillary beds.

(b) Lipolytic Activity of Lung Tissue (Fig. 4). The lipolytic activities of the right lung (unperfused) and the left lung (perfused) were higher in force-fed dogs than in fasted dogs but in both the activities were reduced in comparison with the intact one. After perfusion, the activities of both the lungs were reduced and the marked reduction of the activity was seen in force fed dogs. This reduction of the activity might be due to release of lipolytic enzyme into perfusion fluid.

(c) Plasma Lipolytic Activity (Fig. 7). The lipolytic activity of the fasted dog plasma became maximum between five to ten minutes

Fig. 7 Plasma Lipolytic Activity (Group 2)



after the injection of heparin, and decreased about a half of maximum value in 20 minutes after the injection in comparison with intact force fed dogs. The disappearance of the activity was retarded and maximum value was about two thirds of the intact dogs.

(F) *Release of Lipoprotein Lipase in Dextran Infused Dogs (Group 3)*

(a) The Lipolytic Activity of Perfusion Fluids (Fig. 8). The lipolytic activity of perfusion fluid was present before heparin was introduced, thus differing from them in group 1 and 2. Five minutes after adding heparin into the perfusion fluid, the activity increased significantly and the maximum value was highest in three groups. In 60 minutes after the starting of perfusion, the activity remained. The effect of ethyl-linoleate was also observed.

(b) Lipolytic Activity of Lung Tissue (Fig. 4) In the right lung from fasted dogs, the lipolytic activity was about equal to that of normal healthy force fed dogs. In the left lung, the activity of force fed and fasted dogs was decreased to about half of the right lung.

(c) Plasma Lipolytic Activity (Fig. 9) After the starting of dextran infusion, the lipolytic activity of plasma was observed on the interval of 10 minutes till 60 minutes, because dextran (high molecular weight) itself has the heparinlike effect and the action of release of lipoprotein lipase. The maximum level was about 1/10 times lower than that of normal dogs. 60 minutes after the perfusion, the activity disappeared. The effect of ethyl-linoleate was not seen. This may be caused by dilution of plasma from dextran infusion.

(G) *Free Fatty Acid Production from the Lung*

After the starting of the perfusion, free fatty acid contents increa-

Fig. 8 Lipolytic Activity of the Perfusion Fluid (Group 3)

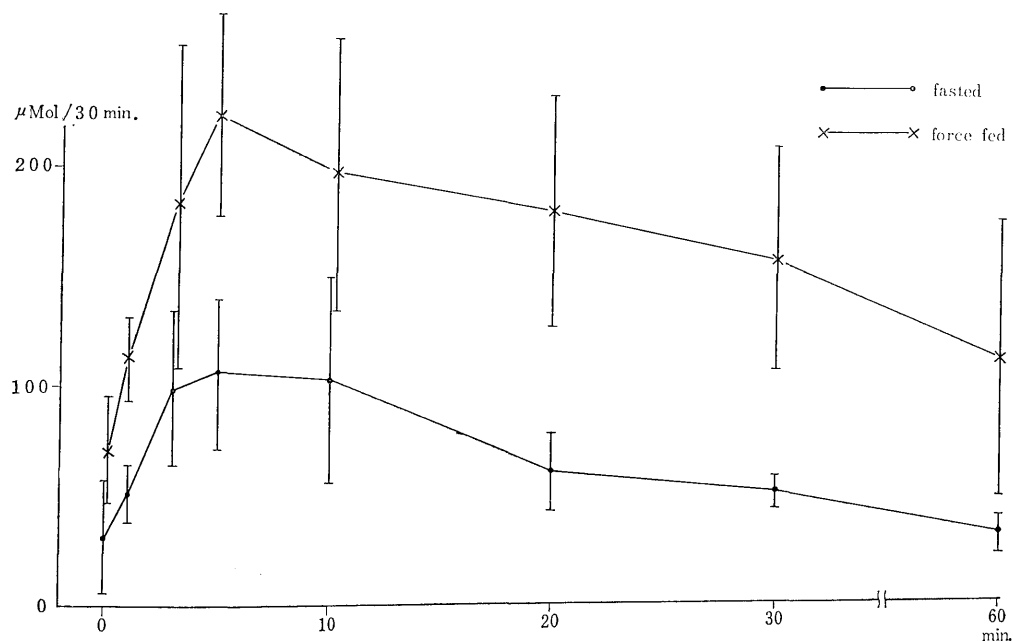
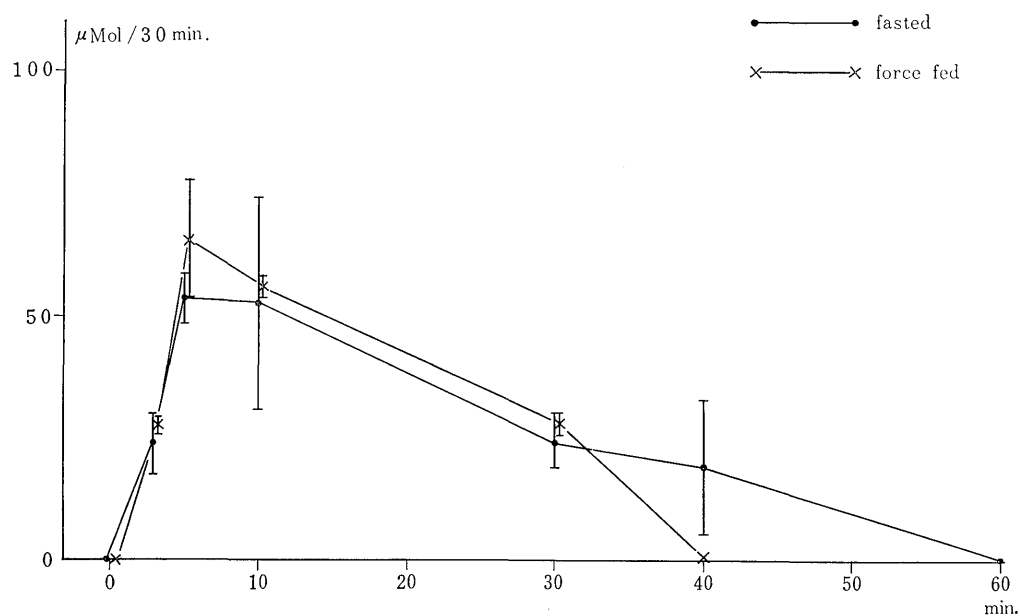


Fig. 9 Plasma Lipolytic Activity (Group 3)



sed in all three groups. In the lungs of force fed intact animals, the production of free fatty acids was somewhat elevated (Fig. 10). In animals injected with clots, free fatty acid production was not so high as that of normal animals (Fig. 11). In dextran infused animals, free

fatty acid production was markedly increased, and administration of ethyl-linoleate produced an increase of free fatty acid (Fig. 12).

Fig. 10 Free Fatty Acid Production through the Lung (Group 1)

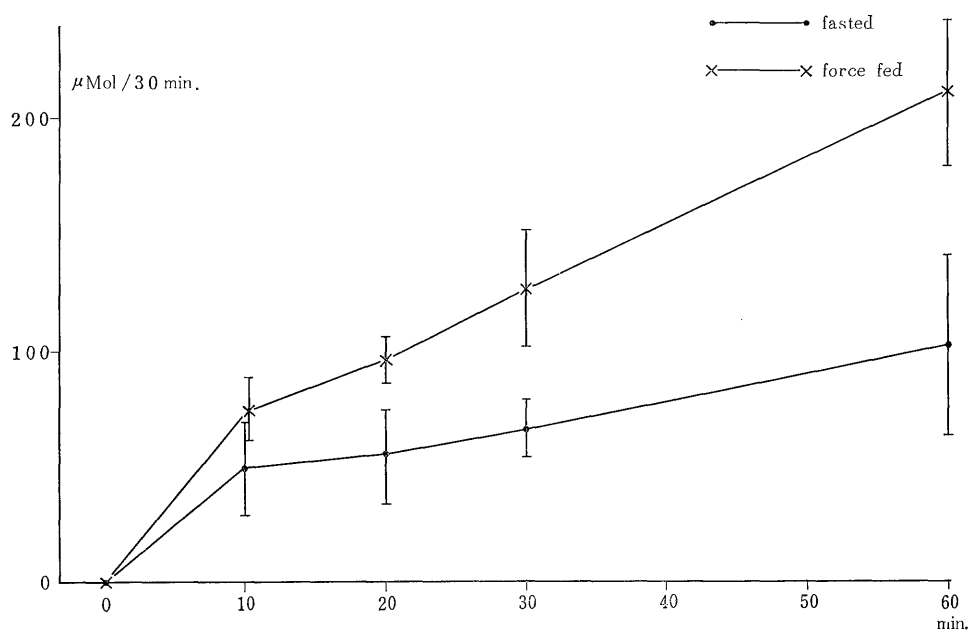


Fig. 11 Free Fatty Acid Production through the Lung (Group 2)

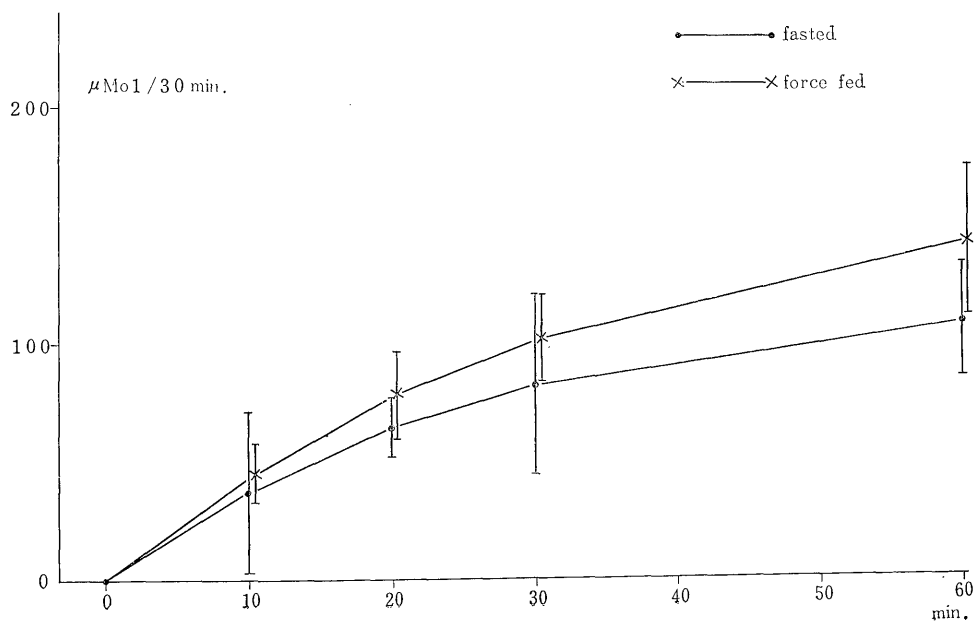


Fig. 12 Free Fatty Acid Production through the Lung (Group 3)

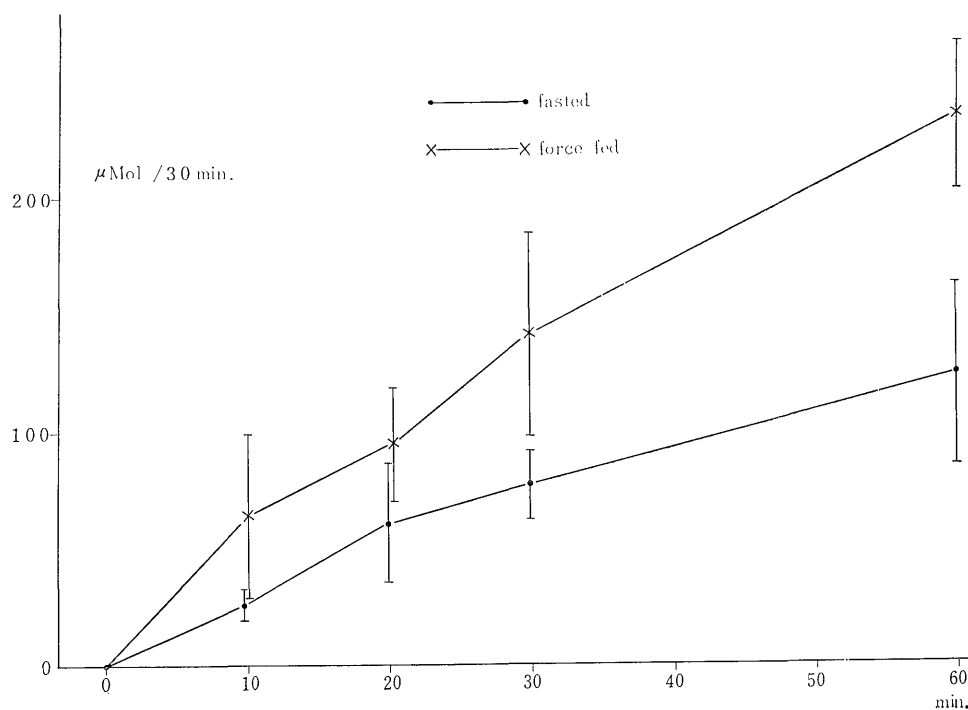


Table 3
Lipid Composition of Right Lung (before Perfusion)

	Total Lipid	Phospholipid	Triglyceride	Total Cholesterol
fasted				
Group 1	68.5 [*] ± 3.2 ^{**}	41.1 ± 8.7	4.3 ± 0.7	15.8 ± 4.4
Group 2	72.9 ± 6.3	34.7 ± 1.8	2.9 ± 1.0	21.9
Group 3	37.5 ± 14.2	27.3 ± 11.9	1.5 ± 0.8	7.4 ± 4.7
force fed				
Group 1	77.3 ± 6.1	56.4 ± 12.0	1.7 ± 0.2	14.8 ± 3.6
Group 2	74.0 ± 5.2	43.2 ± 6.1	2.1 ± 0.8	17.2 ± 1.4
Group 3	39.8 ± 11.1	28.5 ± 9.6	1.3 ± 0.4	11.5 ± 3.9

* mg/2.5 g of wet weight

** standard deviation

(H) Lipid Composition of Right Lung (Table 3)

In force fed dogs of group 1 and 2, especially in group 1, the amount of total lipid increased and that of group 3 was markedly reduced.

In the amount of phospholipid of force fed animals, the marked increase was shown in group 1 and 2. The amount of total cholesterol increased in group 2. The triglyceride contents of force fed animals was reduced in group 1. The increase of the amount of total lipid in force fed animals of group 1 was caused by the increase of phospholipid and the increase in group 2 was due to the increase of total cholesterol.

(I) *Lipid Composition of Left Lung (Table 4)*

Table 4
Lipid Composition of Left Lung (after Perfusion)

	Total Lipid	Paospholipid	Triglyceride	Total Cholesterol
fasted				
Group 1	65.2 [*] ± 5.6 ^{**}	43.6 ± 4.8	3.9 ± 1.2	17.1 ± 4.5
Group 2	63.8 ± 4.9	32.7 ± 2.7	3.7 ± 0.9	22.7 ± 3.0
Group 3	35.5 ± 6.4	23.6 ± 7.2	1.1	6.2 ± 0.9
force fed				
Group 1	59.3 ± 9.5	41.2 ± 5.4	2.9 ± 1.1	17.6 ± 3.0
Group 2	60.8 ± 6.3	26.9 ± 4.2	2.8 ± 1.0	13.7 ± 2.2
Group 3	35.5 ± 4.9	22.6 ± 2.5	1.5 ± 0.4	7.2 ± 0.8

* mg/2.5 g of wet weight

** standard deviation

The amount of total lipid decreased in all groups except for the fasted animals of group 1. The decrease of the amount of phospholipid was observed in the force fed animals of the three groups. The amount of total cholesterol was reduced in the force fed animals of group 2 and 3, in comparison with that of fasted animals. The amount of triglyceride did not show any remarkable changes in each group 1 and 2.

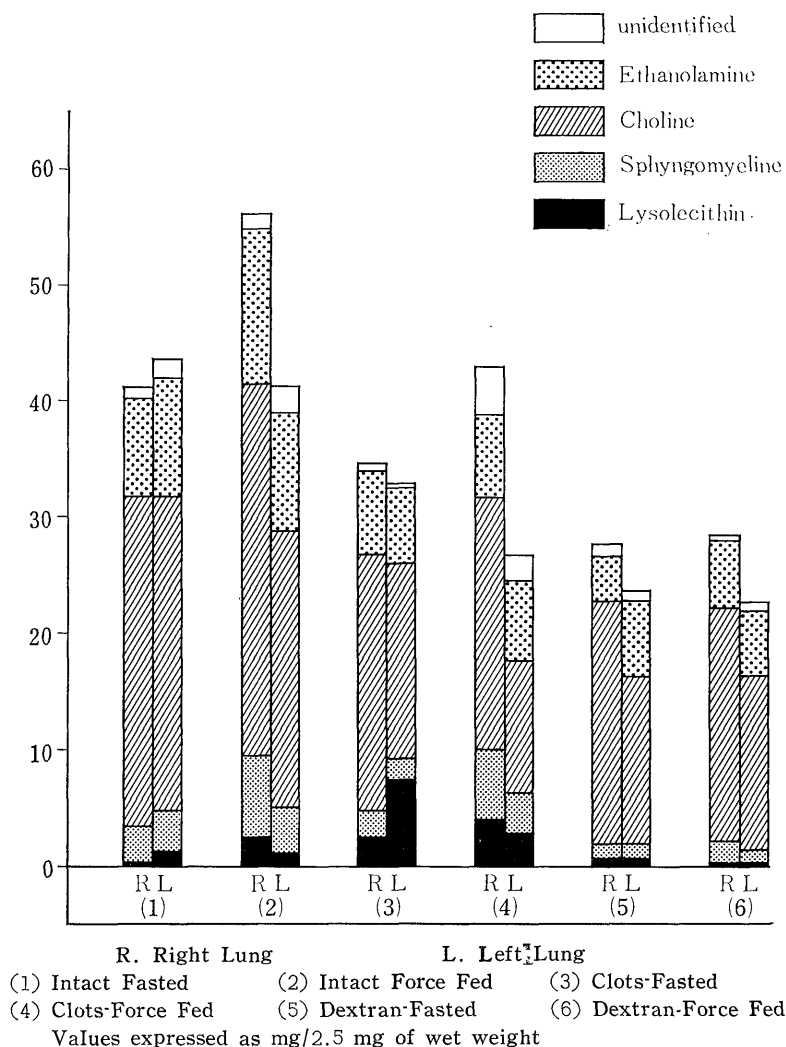
(J) *Proportion of Phospholipid Subfraction (Fig. 13)*

By thin layer chromatographic technique, the lung phospholipid was separated into five subfractions, which were consisted of phosphatidylcholine, ethanolamine, sphingomyelin, lysolecithin and an unidentified spot. More than 50 % of the amount of phospholipid was occupied by choline fraction. The decrease of the phospholipid contents in the force fed dogs extending from group 1 to 3, was revealed in the reduction of the choline or ethanolamine fraction. The other subfractions of phospholipid did not show any remarkable changes.

(K) *Fatty Acid Composition of Choline*

The results of group 1 (fasted dogs) and group 3 are shown in Fig. 14 (a) (b).

Fig. 13 Proportion of the Amount of Phospholipid Subfraction



The concentration of the saturated fatty acid (16:0 and 18:0) decreased and the unsaturated fatty acid (16:1, 18:1, 18:2, 18:3 and 20:4) increased in the right (unperfused) dextran infused dog lung. The remarkable change was noted in the dextran infused force fed dog lungs (Fig. 14-a). The changes of the concentration of the saturated fatty acid in the left (perfused) dextran infused dog lung decreased similarly to the findings in the right lung (Fig. 14-b).

DISCUSSION

In this study, it was demonstrated that the normal dog lung had a lipolytic activity. From force fed dogs, the lipolytic activity of perfusion fluids was higher than that of fasting dogs. The lipolytic activity of lung

Fig. 14 (a) Fatty Acid Composition of Choline (Right Lung Tissue)

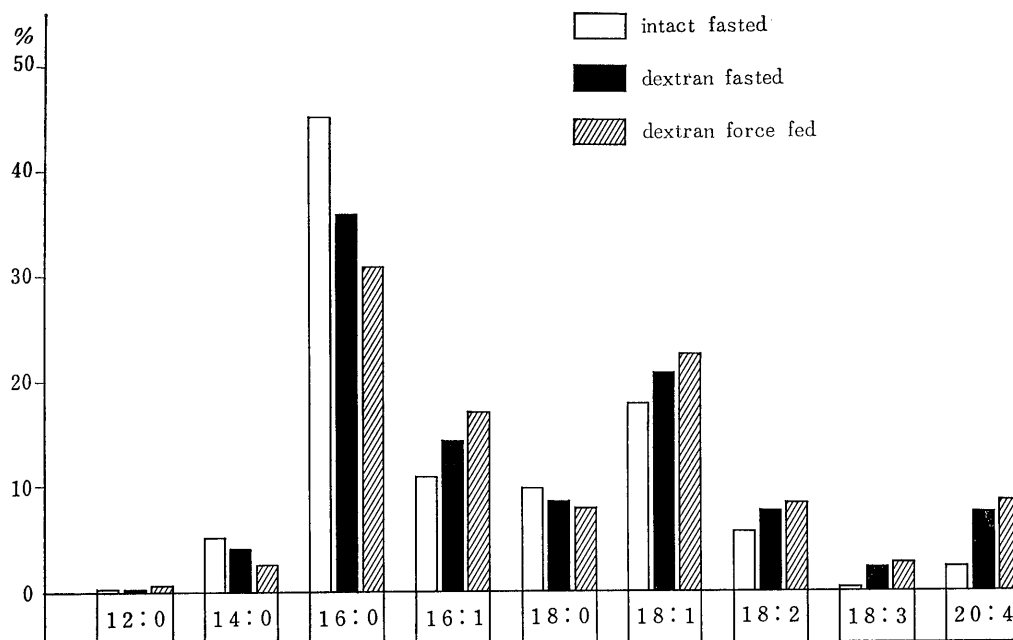
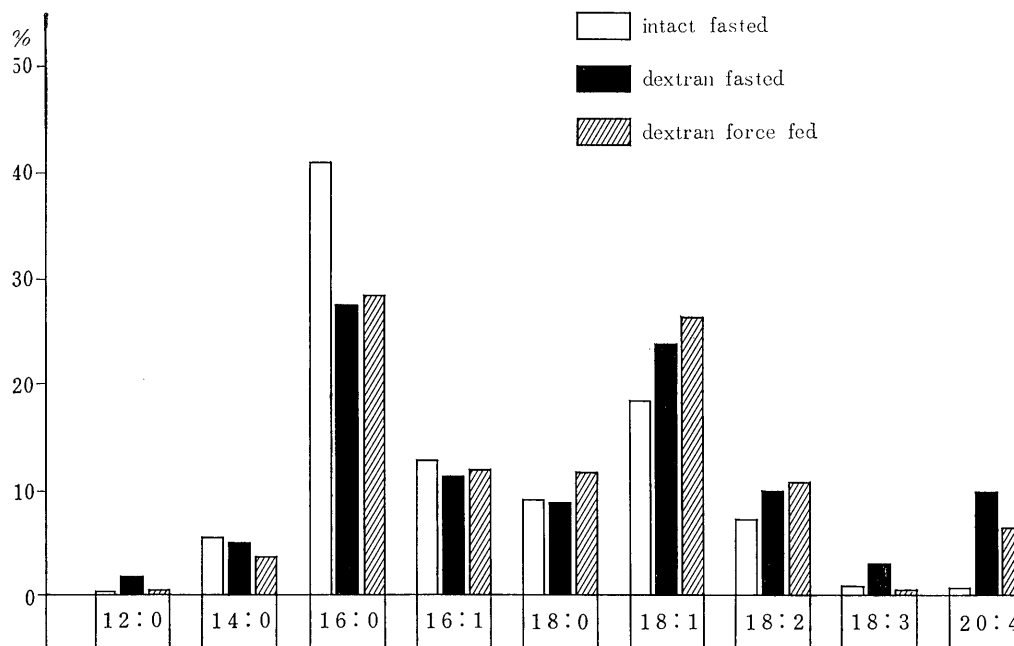


Fig. 14 (b) Fatty Acid Composition of Choline (Left Lung Tissue)



tissue was decreased after perfusion and the decreasing rate was marked in the force fed postperfusion lung. Plasma lipolytic activity increased in the force fed animals. In 1943, HAHN¹³ observed that heparin

caused rapid clearing of alimentary lipemic plasma *in vivo* but that this decrease in turbidity did not occur *in vitro*. It has been demonstrated by many investigators that a lipolytic enzyme appeared in the blood rapidly after the injection of heparin, and the enzyme was found only in curde form. E.D. KORN⁸⁾ demonstrated no lipolytic enzyme in lung acetone powder extracts. On the otherhand, it was demonstrated, many years ago, from the work of HAMSIK and others⁴⁰⁾ that this tissue contained lipolytic enzymes. ZEMPLÉNYI and GRAFNETTER¹⁶⁾ demonstrated the lipolytic activity of the lung from some species and SAILER et al⁴¹⁾ reported similar results. HEINEMANN¹⁸⁾ reported that the fresh tissue showed no change of lipolytic activity but the acetone extracted tissue had activity, and that the plasma lipolytic activity was not changed by perfusion through the lung. JEFFRIES⁴²⁾, however, observed in rats the appearance of lipolytic activity in the perfusion of heparin containing blood through the lung (and also in the isolated hind limb, the abdominal viscera, the skin and the subcutaneous tissue). In this study, the plasma lipolytic activity was higher in force fed animals than that in fasted animals. The lung tissue lipolytic activity increased in force fed animals and after perfusion, the activity decreased markedly. This fact suggested that the lipoprotein lipase localizing in the pulmonary capillary wall was released into the perfusion fluids, because the elevation of the lipolytic activity of the perfusion fluids continued until 60 minutes after the starting of the perfusion. Many studies^{27), 43), 44), 45), 46), 47)} have been carried out in experimental pulmonary fibrosis or arteriosclerosis. In rabbits, the repeated intravenous injection of small fibrinous clots from human blood led to fibroelastic intimal thickenings of the pulmonary arterioles⁴³⁾. In another experiment in rabbits, the intravenous injection of minute fibrin emboli from rabbit's own blood caused an acute inflammatory arteritis, and also interstitial pneumonia. The fibrin clots were organized into the pulmonary arterial wall and these findings were similar to the human primary pulmonary arteriosclerosis, and not to atherosclerosis²⁷⁾. In the clinical state, arteriosclerotic patients have ordinarily hyperlipemia supporting the relationship between arteriosclerosis and lipoprotein lipase activity. There is a report that the endogenous lipoprotein lipase activity in fasting human plasma was slightly reduced⁴⁸⁾. Another report proved that there was a difference between healthy and arteriosclerotic patients in the postheparin plasma lipoprotein lipase activity. It has also been noted that arteriosclerotic patients have an inhibitor of the postheparin clearing factor. ENGERBERG reported that the hyperlipemia might be caused by saturated, but not by unsaturated fatty acids and so saturated fatty acids were hardly effected by lipoprotein lipase⁴⁹⁾. In this study, the lung fibrosis was produced in dogs by the injection of fibrin clots. Plasma lipolytic activity was reduced in fasted and force fed dogs in comparison with that of normal healthy dogs. From fasted

dogs, the lipolytic activity of dog lungs injected with clots was not different from that of normal healthy fasted dogs but from force fed dogs, the former was markedly reduced. This fact agreed with the reports which the lipoprotein lipase might be liberated from the cell walls to the circulating blood by the action of heparin, and suggested the alteration of the pulmonary capillary bed led to the reduction of the production or to some change of the releasing mechanism of lipoprotein lipase from the capillary bed. The relationship between heparin and lipoprotein lipase has been discussed for many years. ANFINSEN et al⁵⁰⁾ suggested that heparin was a part of the composition of lipoprotein lipase. Protamin and basophilic pigments which have the ability to combine with heparin, inhibit the activity of lipoprotein lipase. SPITZER and SPITZER⁵¹⁾ observed that the destruction of postheparin lipoprotein lipase occurred by the hepatic heparinase and KORN⁵²⁾ reported that tissue lipoprotein lipase was inactivated by heparinase of some bacteria, and suggested that heparin was a part of an essential component of lipoprotein lipase. Dextran, phosphoribudate and tungstate, and other sulphated polysaccharides of high molecular weight have heparinlike effect and all these substances are highly polar and carry a strong negative charge. Also in this study, the lipolytic activity of the perfusion fluids after the injection of dextran was markedly increased and lipolytic activity of the lung tissue was markedly lowered from the other group 1 and 2. HEINEMANN¹⁸⁾ demonstrated experimentally that free fatty acid was released from the rabbit lung when perfused with the exogenous triglycerides and that rabbit lung had a lipoprotein lipase which was due to the pulmonary vasculature and not to that of the plasma. The free fatty acid from the hydrolysis of triglycerides was supplied to some tissues for the utilization as energy source. Furthermore, LOCHNER and NASSERI⁵³⁾ observed that free fatty acid concentration from the arterio-venous blood of dogs was higher than that of simultaneously collected venous blood. In this experiment, the increase of free fatty acid was demonstrated in the perfusion fluid in the course of time, and the increasing rate was lowered in the lung from clots injected dogs but elevated mostly in the lung from dextran injected dogs. The explanation of the difference of the activity between dextran and heparin is difficult. The pulmonary surfactant has been studied by dextran induced lung edema by many investigators. Mammalian lung alveolus is lined by a thin film composing the air-liquid interface of alveolus, reduces the surface tension of the alveolus, and has the function of preserving the stability of fine air space during respiration. This specific surface active agents has been called pulmonary surfactant. PATTLE and THOMAS⁵⁴⁾, and CLEMENTS⁵⁵⁾ suggested that this material was a lipid-protein complex and the predominant lipid was lecithin. From a clinical standpoint, the changes in surface tension has been studied in hyaline membrane disease^{56), 57), 58)} postperfusion syndrome⁵⁹⁾ and oxygen intoxica-

tion^{60), 61)}. KLAUS, CLEMENTS and HAVEL⁶²⁾ found that the extracts of surface active materials isolated from beef lungs contained 74% phospholipids, 8% cholesterol, 10% triglyceride and 8% fatty acid and only the phospholipid fraction had a marked surface tension activity while cholesterol, triglyceride and fatty acid had no activity. BROWN⁶³⁾ demonstrated that palmitic acid increased in the fatty acid composition of lecithin. FINLEY, MORGAN and FIALKOW⁶⁴⁾ reported that distinct differences in the amount of lecithin between dog lung homogenate and lung wash was present. They also showed that the amount of phospholipid decreased, and that lecithin in the phospholipid fraction contained increased amounts of esterified palmitate and that unsaturated fatty acid was lower following the absence of arachidonic acid in the lung after ligation of the pulmonary artery. NAIMARK^{65), 66)} reported that the radioactivity appeared in the lung tissue and 40% of the activity was in the phospholipid fraction one hour after the intravenous injection of palmitate-1-C¹⁴ in dog and in experimental atelectasis, the activity was reduced. Furthermore, NAIMARK et al⁶⁷⁾ reported that about 50% of the total lipid activity appeared in phospholipid, about 25% in triglyceride, and most of the remaining activity in the free fatty acid fraction in the study of the incorporation with palmitate-1-C¹⁴ by rat lung in vitro. FELTS⁶⁸⁾ suggested some metabolic pathway in the alveolar cell concerned with circulating lipids that free fatty acid enters the cell directly from the plasma, from hydrolysis of plasma triglycerides by the effect of lipoprotein lipase or are synthesized within the cell from acetate. The Co-enzyme A derivatives of free fatty acid may be oxidized to CO₂ or esterificated to triglyceride and phospholipid depending on the availability of L- α -glycerol phosphate derived from glucose. KLAUS et al⁶⁹⁾ and SAID⁷⁰⁾ reported that pulmonary surfactant might be synthesized in the alveolar cell mitochondria. On the otherhand, TOMBOROPOULOS⁷¹⁾ discussed that the free fatty acid might be synthesized in the same subcellular fractions. In this study, the phospholipid content was markedly reduced in group 3. The amount of phosphatidyl choline was reduced in the force fed dogs of group 1 and 3. This change was marked in the dextran infused force fed dogs. These findings were obtained in the right (unperfused) and the left (perfused) lungs in the same way. From the gas liquid chromatographic analysis of phosphatidyl choline, the saturated fatty acid especially palmitic acid decreased and the unsaturated fatty acid increased in the dextran infused lung tissue. This fact may suggest the loss of surfactant of the dextran infused lungs. Lipoprotein lipase from dextran infused postperfused lung tissue decreased markedly in comparison with control and clots injected groups. This fact may suggest the relationship between the free fatty acid production of lipoprotein lipase and synthesis of pulmonary surfactant and thus the lung also performs active metabolic functions.

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